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IODOTHYRONINE 5-DEIODINASE IN RAT POSTERIOR PITUITARY

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Summary We first describe the presence of iodothyronine 5-deiodinase (5D) in the neural lobe of rat pituitary. 6-n-Propyl-2-thiouracil (PTU), a specific inhibitor of type-I deiodinase, had no effect, showing that 5D in neurohypophysis is of type-III isozyme, which is specific for 5-deiodination and has been found only in the brain, placenta and skin. The presence of 5D (type-III) together with our previous report of 5'-deiodinase (type-I in euthyroidism and type-II in hypothyroidism) shows that the isozymes of deiodinases in the neurohypophysis are quite similar to those in the brain. These data suggest a previously unrecognized role of thyroid hormone in posterior pituitary physiology.

Thyroxine (T_4) is a prohormone and must be metabolized to 3,5,3'-L-triiodothyronine (T_3) by 5'-deiodinase (5'D) to exert its hormone action. T_4 is also converted to hormonally inactive isomer of T_3, 3,3',5'-L-triiodothyronine (reverse T_3, rT_3) by 5-deiodinase (5D). 5'D consists of at least two isozymes; i.e. type-I (5'D-I) and type-II (5'D-II). The latter is characterized by its high affinity for T_4, and is present in only a limited number of tissues. True 5D (type-III), specific for 5-deiodination, has been reported only in the brain, placenta and skin (for review, 1-4). In addition to our previous report of 5'D in rat posterior pituitary (5), we decribe the presence of 5D (type-III) in this paper.

Materials and Methods

Seven-week-old male Wistar rats were kept under 12-hours light and dark cycle (06:00 to 18:00) with free access to water and Oriental Rat Chow at least one week before use.

Abbreviations: T_4; L-Thyroxine, T_3; 3,5,3'-L-triiodothyronine, rT_3; 3,3',5'-L-triiodothyronine, 3,3'-T_2; 3,3'-L-diiodothyronine, 3'-T_1; 3'-L-iodothyronine, DTT; DL-dithiothreitol, PTU; 6-n-propyl-2-thiouracil, 5D; 5-deiodinase, 5'D; 5'-deiodinase, 5'D-I; type-I 5'-deiodinase, 5'D-II; type-II 5'-deiodinase. NIL; neurointermediate lobe.

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T_4, T_3 and DL-dithiothreitol (DTT) were purchased from Sigma (St. Louis, MO); rT_3 from Calbiochem (San Diego, CA). [3'-^{125}I]T_3 (>1200mC$_i$/mg) and Na^{125}I were purchased from Amersham Japan (Tokyo); [3'-^{125}I]3,3'-L-diiodothyronine (3,3'-T_2) and [3'-^{125}I]3'-L-iodothyronine (3'-T_1) from Hungarian Academy of Sciences (Budapest, Hungary). Radioimmunoassay kit for rat β-endorphin (catalog No. RIK-8843) was purchased from Peninsula Lab (Belmont, CA); protein assay kit with a BSA standard, based upon a dye-binding assay (6) from Pierce (Rockford, IL).

Rats were decapitated around 11:00. Neurointermediate lobes (NIL) were removed and homogenized in ice-cold 100 mM phosphate buffer, pH 7 containing 20 mM DTT (50 µl/lobe).

In some experiments, neural lobes were freed from intermediate lobes under stereomicroscope with fine forceps and intravenous needles (5). Possible contamination of intermediate lobes into neural lobe preparations was evaluated by measuring the β-endorphin contents in acetic acid extracts of these lobes (7), since β-endorphin in NIL is localized to intermediate lobe (8).

Forty µl of the homogenates were incubated with 10 µl [^{125}I]T_3 (approximately 50,000 cpm/tube) at 37C for 60 minutes, followed by extraction by 2 volumes of ethanol. After storage at -20C overnight, the extracts were centrifuged at 3,000 rpm for 15 minutes. The supernatants were evaporated to dryness under N_2 stream, disssolved in acetonitrile/water (32.5:67.5), applied to HPLC (Shimadzu LC-9A with CLC-ODS(M) C_{18} column, 4.6 x 150 mm) and eluted with acetonitrile/water/phosphoric acid (32.5:67.5:0.1)(9). The flow rate was 1 ml/min. Each 0.5 min fraction was collected and measured for radioactivity by an automated gamma-counter. 5D activity was expressed as fmol 3,3'-T_2 producted/mg protein/hr. Data are means of duplicate determinations.

Results

An example of HPLC chromatogram is shown in figure 1. Only two peaks corresponding to T_3 and 3,3'-T_2 were appreciable. Retention time for I^-, 3'-T_1, 3,3'-T_2 and T_3 were 2.5 min, 3 min, 5 min and 7 min, respectively. 5'-deiodination of T_3 or further degradation of 3,3'-T_2 was unlikely because there was no peak at I^-/3'-T_1 position.

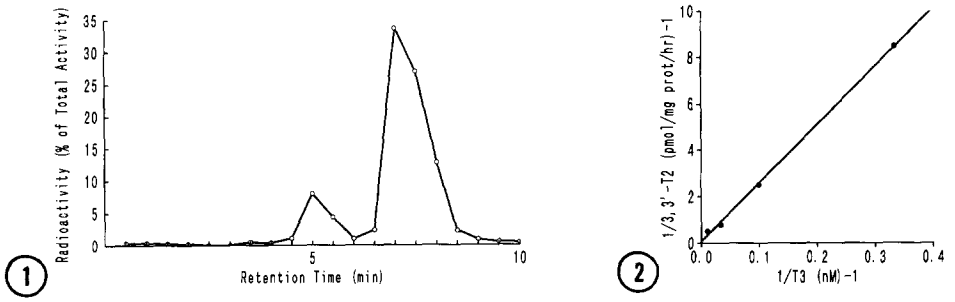
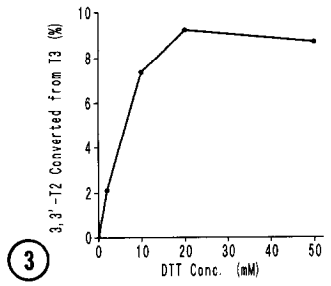


Figure 1. An example of HPLC chromatogram (Shimadzu LC-9A, CLC-ODS(M) C_{18} column, 4.6x150 mm). Ethanol extracts of the incubaion mixture were evaporated and were dissolved in HPLC solvent. Twenty µl was applied and eluted with acetonitrile/water/phosphoric acid (32.5:67.5:0.1) and each 0.5 min fraction was collected. Retention times for I^-, 3'-T_1, 3,3'-T_2 and T_3 were 2.5 min, 3 min, 5 min and 7 min, respectively.

Figure 2. Double reciprocal plot of T_3 to 3,3'-T_2 conversion. Each point is the mean of duplicate determinations.



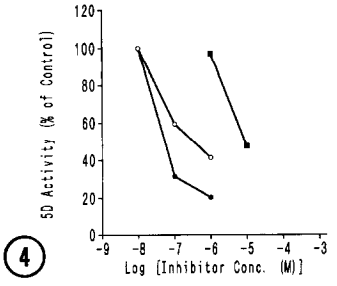


Figure 3. DTT dependence of T_3 5-deiodination. Each point shows the mean of duplicate determinations.

Figure 4. Effect of non-labeled T_3 (●), T_4 (○), RT_3 (■) and PTU (◆) on 5-deiodination of a tracer amount of [^{125}I]T3 in the posterior pituitary. Each point shows the mean of duplicate determinations.

3,3'-T_2 production from [^{125}I]T_3 was dependent on tissue amount and incubation period. Typically the incubation mixture contained 40-70 µg protein and was incubated for 60 min. Under these conditions, 3,3'-T_2 production rate was 8-12 %.

The reaction was dependent on the pH of the incubation mixture. T_3 5-deiodination was optimal at pH 7-7.5. From the double reciprocal plot, shown in figure 2, K_m and V_{max} were calculated to be 311 nM and 12 pmol/mg prot/hr, respectively. The deiodination was dependent on DTT for its activity (figure 3).

Table 1 T_3 5-deiodinase activity (5D) and β-endorphin content in rat pituitary gland

		5D (U/gland)	5D (U/mg prot)	β-endorphin (ng/gland)
Experiment A				
Anterior lobe	(3)		15.9 ± 1.7	
Posterior lobe	(3)		235.8 ± 68.9	
Experiment B				
NIL	(4)	11.2 ± 0.0	120.7 ± 14.1	512.9 ± 45.3 *
Neural lobe	(4)	9.3 ± 0.7	157.4 ± 22.9	68.0 ± 19.4

Data are mean ± SEM from three or four determinations, as individually indicated in parentheses. One unit is arbitrarily defined as fmol 3,3'-T_2 production/hr.
NIL is an abbreviation of neurointermediate lobe. *; $p<1\%$ compared to neural lobe.

The effects of iodothyronine analogs and PTU on T_3 5D were studied. Percentage of [^{125}I]3,3'-T_2 converted from a tracer amount of [^{125}I]T_3 (subnanomolar concentration) was suppressed by non-radioactive T_4 and T_3. RT_3 was less potent and PTU at 1mM had no efffect (figure 4).

5D activity in the anterior pituitary was also measured for comparison. As shown in table 1 (experiment A), it was far lower than that in the posterior pituitary.

Finally 5D was measured in whole NIL and neural lobe. As shown in table 1 (experiment B), approximately 80% of 5D in NIL was recovered in neural lobe. When corrected for protein content, 5D in neural lobe was rather higher than that in whole NIL. β-Endorphin levels in neural lobe were approximately 13% of that in NIL.

Discussion

Most studies on thyroid hormone metabolism has been directed to 5'-deiodinase (5'D). 5-Deiodinase (5D) has received far less attention. Although type-I isozyme mainly catalyzes 5'-deiodination and is often abbreviated as 5'D-I, hepatic and renal 5-deiodination is considered to be also mediated by 5'D-I because of some wobble in their active center (1-4). An isozyme, specific for 5-deiodination (Type-III) has been reported only in the brain (10-11), placenta (12) and skin (13). It is characterized by its relative insensitivity to 6-propyl-2-thiouracil (PTU) inhibition, which is a specific inhibitor of 5'D-I.

In this paper we described the presence of 5D in rat posterior pituitary for the first time. Dependence on protein content, incubation period, pH and substrate concentration were compatible with its enzymatic nature. 5D activity was not affected by 1 mM PTU in the presence of 20 mM DTT, under which condition type-I isozyme is completely inhibited (1-4). Thus, 5D in the posterior pituitary is likely to be of type-III isozyme. Requirement of high concentration of DTT for its full activity and apparently higher affinity for T_4 and T_3 over rT_3 are also compatible with this isozyme.

K_m value in the present study is higher than that reported by Kaplan for cerebrocortical type-III deiodinase (5.5 nM for T_3 and 37 nM for T_4) (10). Chopra

reported the K_m's for T4 in cerebrocortical and skin type-III 5D to be 36 nM and 290 nM, respectively (13). These discrepancies are likely to be due to the differences in the assay procedure and tissues employed. For example, cerebrocortical microsomes were used in Kaplan's work (10). However, it is practically impossible to obtain the microsomes from rat posterior pituitary and compare the Km values, because of the limited amount of the tissue. Further studies are necessary on the kinetic parameters of type-III 5D.

Approximately 80% of 5D in NIL was recovered in the neural lobe. The enzyme activity per mg protein was rather higher in neural lobe than that in whole NIL. Removal of intermediate lobe seemed satisfactory, since almost 90% of β-endorphin in NIL was removed by our procedure. Thus we conclude that most, if not all, of 5D in rat NIL is in neural lobe. Whether weak 5D activity found in the anterior lobe implies the presence of 5D in the anterior pituitary or incomplete separation, awaits further studies.

Previously we have found 5'D in rat neurohypophysis (5). The predominant isozyme was type-I in euthyroidism and type-II in hypothyroidism. In this paper we described type-III 5D in the neural lobe. Rat brain contains both 5'D (type-I in euthyroidism and type-II in hypothyroidism) and 5D (type-III) (1-4,14). Thus thyroid hormone metabolism in neurohypophysis is quite similar to that in the brain.

Our data have at least two implications. First, the presence of these deiodinases suggests a previously unrecognized role of thyroid hormones in the posterior pituitary. For eample, it may be related to the pathogenesis of SIADH (syndrome of inappropriate secretion of antidiuretic hormone) in hypothyroidism, or it may influence adenohypophyseal function (5). Second, neurohypophysis can be a simplified model to study the regulatory mechanism of the deiodinases in the brain, because it is composed of only two components; i.e. nerve ending from the hypothalamus and pituicyte which is similar to astrocyte in the brain.

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